Cytoplasmic dynein-2 at-a-glance

Laura Vuolo[1], Nicola L. Stevenson[1], Aakash G. Mukhopadhyay[2], Anthony J. Roberts[2], and David J. Stephens[1]*

[1] Cell Biology Laboratories, School of Biochemistry, Faculty of Life Sciences, University of Bristol, BRISTOL, BS8 1TD, UK.

[2] Institute of Structural and Molecular Biology, Birkbeck, University of London, London, UK.

*Author for correspondence (david.stephens@bristol.ac.uk)

ORCID:

L.V., 0000-0002-9801-9206

N.L.S., 0000-0001-8967-7277

A.G.M., 0000-0001-9397-9702

A.J.R., 0000-0001-5277-6730

D.J.S., 0000-0001-5297-3240

Abstract

Cytoplasmic dynein-2 is a motor protein complex that drives the movement of cargoes along microtubules within cilia, facilitating the assembly of these organelles on the surface of nearly all mammalian cells. Dynein-2 is critical for ciliary function as evidenced by deleterious mutations in patients with skeletal abnormalities. Long-standing questions include how the dynein-2 complex is assembled, regulated, and switched between active and inactive states. A combination of model organisms, *in vitro* cell biology, live-cell imaging, structural biology, and biochemistry has advanced our understanding of the dynein-2 motor. In this Cell Science at the Glance and the accompanying poster, we showcase current understanding of dynein-2 and its roles in ciliary assembly and function.

KEY WORDS: dynein-2, cilia, intraflagellar transport, microtubule motors

Introduction

Cytoplasmic dynein-2 (here “dynein-2) is an ATP-dependent motor protein that steps along microtubules to transport cargoes within cilia and flagella (Box 1). It is related to cytoplasmic dynein-1 (here “dynein-1”), which is involved in the transport of cargos within the cytoplasm, organelle dynamics (Reck-Peterson et al., 2018), and mitotic spindle organization during mitosis (Raaijmakers and Medema, 2014). In contrast, dynein-2 does not act in canonical membrane traffic (Palmer et al., 2009), but functions primarily, if not exclusively, within the intraflagellar transport (IFT) system (Box 2). Here, dynein-2 assembles with kinesin-2, IFT-A complexes, and IFT-B complexes to form polymeric IFT “trains”, which move cargoes to the ciliary tip (kinesin-2 direction) and back to the cell body (dynein-2 direction). Dynein-2-driven transport occurs in the confined space between the ciliary microtubule doublets and the ciliary membrane (Roberts, 2018). There is some evidence for dynein-2 functions outside of cilia; for example, in *Chlamydomonas*, which lacks dynein-1, dynein-2 is implicated in cytoplasmic trafficking to the base of cilia (Cao et al., 2015).

Dynein-1 and dynein-2 are distantly related to their axonemal cousins (Kollmar, 2016; Wickstead and Gull, 2007), which drive the beating of motile cilia and flagella (Box 1). Below, and in the accompanying poster, we provide an overview of dynein-2 discovery, subunit composition, structure, and regulation. We also discuss new insights into the functions of dynein-2 in mantaining the ciliary transition zone – the gatekeeper between the cilium and the cytoplasm (Box 1) – as well as the connection between dynein-2 and human disease.

Discovery of dynein-2 and its role in IFT

Dynein-2 was first identified in sea-urchin (Gibbons et al., 1994) and rat (Tanaka et al., 1995) based on sequence similarity to dynein-1 In mammals. It was described as a cytoplasmic dynein and shown to be upregulated prior to ciliogenesis in sea urchin embryos (Gibbons et al., 1994) and mammalian cells (Criswell et al., 1996). Retrograde IFT was first linked to a cytoplasmic dynein motor in *Chlamydomonas* (Pazour et al., 1998). Further work revealed that mutations in dynein-2 resulted in cells with short flagella that accumulated IFT proteins at their tip (Pazour et al., 1999b; Pazour et al., 1998; Porter et al., 1999), and also perturbed retrograde transport of kinesin-2 in *C. elegans* (Signor et al., 1999).

Structure and composition of dynein-2

Dynein-2 is a large multiprotein complex, composed of 16 copies of at least eight different proteins in humans (see poster). Insights into dynein-2 subunit composition have come from a variety of cell biology, genetic, and biochemical studies (see below), and a recent cryo-EM structure of the dynein-2 complex (Toropova et al., 2019). Like other dyneins, the subunits of dynein-2 are classified as heavy chains, intermediate chains, light-intermediate chains, and light chains depending on their mass. Most subunits in the dynein-2 complex are unique to dynein-2, but a subset of the light chains are also found in dynein-1 (Asante et al., 2014). Naming of dynein-2 subunits varies (see poster) and here we use the human nomenclature unless specified.

Dynein-2 is built around two copies of the heavy chain, DYNC2H1 (Criswell et al., 1996; Mikami et al., 2002). The C-terminal region forms the motor domain, which converts the energy from ATP hydrolysis into movement (Schmidt et al. 2015). The N-terminal region forms the tail: an extended structure that binds the other subunits (Hamada et al., 2018) and holds the two heavy chains in a homodimer (Toropova et al., 2017; Toropova et al., 2019). In an interesting variation compared to other organisms, trypanosomatids possess two distinct dynein-2 heavy chains that form a heterodimer (Adhiambo et al., 2005; Blisnick et al., 2014).

The dynein-2 light-intermediate chain, DYNC2LI1 (Grissom et al., 2002; Hao et al., 2011; Hou et al., 2004; Li et al., 2015; Mikami et al., 2002), binds directly to the tail of each heavy chain and is important for stabilising its structure (Hou et al., 2004; Reck et al., 2016; Toropova et al., 2017). The light-intermediate chain has a Ras-like fold and appears to bind to nucleotide (Schroeder et al., 2014; Toropova et al., 2019). Although nucleotide-binding by the light-intermediate chain does not seem essential for dynein-2 function (Hou et al., 2004), whether it serves a structural role or has a minor regulatory function remains unclear.

The other dynein-2 subunits – namely, the intermediate chains and light chains – form an unusual stoichiometry subcomplex at the core of dynein-2's tail, which makes the structure of dynein-2 highly asymmetric (Toropova et al., 2019). While dynein-1 is composed of homodimeric subunits, including its intermediate chain, dynein-2 notably differs in that it contains two different intermediate chains. Originally defined as FAP133 (Rompolas et al., 2007) and FAP163 (Patel-King et al., 2013) in *Chlamydomonas*, these subunits have been validated as *bona fide* mammalian dynein-2 subunits, WDR34 (Asante et al., 2013; Asante

et al., 2014; Huber et al., 2013; Schmidts et al., 2013b) and WDR60 (Asante et al., 2014; McInerney-Leo et al., 2013).

WDR34 and WDR60 form a heterodimer (Asante et al., 2014; Hamada et al., 2018; Toropova et al., 2019; Vuolo et al., 2018) (see poster). Their C-terminal β-propeller domains each bind a copy of the heavy chain, and their extended N-terminal regions are held together by an array of light chain dimers (Toropova et al., 2019). These comprise one DYNLRB dimer, which binds proximal to the β-propellers, followed by three DYNLL dimers, and a putative DYNLT-TCTEX1D2 heterodimer (Asante et al., 2014; Hamada et al., 2018; Kanie et al., 2017; Toropova et al., 2019; Tsurumi et al., 2019). Co-expression studies indicate that WDR34 preferentially interacts with DYNLL and DYNLRB, whereas WDR60 preferentially interacts with DYNLT-TCTEX1D2 (Hamada et al., 2018). Among the light chains, TCTEX1D2 is specific to dynein-2 (Asante et al., 2014; Gholkar et al., 2015; Schmidts et al., 2015). The other light chains (DYNLRB, DYNLL, and DYNLT) are also found in dynein-1 (Asante et al., 2014), and each has two orthologs in mammals (e.g. DYNLRB1 and DYNLRB2). The orthologs appear to play interchangeable roles (Hamada et al., 2018) but may have subtly different biochemical properties or generate tissue-specific expression patterns (King et al., 1998). In summary, the unusual stoichiometry of dynein-2's intermediate and light chains is a distinctive feature of the complex; as described below, it has important roles in dynein-2 motility regulation and attachment to IFT trains.

Regulation and Motility

Dynein-2 motility is tightly regulated to enable its functions in IFT. The dynein-2 motor domain contains a ring of six AAA+ modules, of which the N-proximal module (AAA1) is the main ATPase site (Schmidt et al., 2015). N-terminal to AAA1 is a rod-like 'linker' domain that amplifies conformational changes. Dynein-2's microtubule-binding domain is at the tip of a coiled-coil stalk (see poster).

The current generally accepted model is that dynein-2 is transported passively from the ciliary base to tip by kinesin-2 (Hao et al., 2011; Rosenbaum and Witman, 2002). Following activation, it then actively transports the IFT machinery and cargoes from tip to base during retrograde IFT. The motile properties of the human dynein-2 motor domain have been recently described using *in vitro* assays (Toropova et al., 2017). Interestingly, monomeric constructs moved significantly faster (around 500 nm/s) than dimers, as the motor domains in the dimer stack against one another to give rise to an auto-inhibited

conformation (Toropova et al., 2017; Toropova et al., 2019). Accordingly, disruption of the stacking interface induced a significant increase in velocity. These results suggested that the dynein-2 motor domains intrinsically exist in an autoinhibited, stacked conformation, that facilitates transport of dynein-2 to the ciliary tip by kinesin-2 (Toropova et al., 2017). Supporting this model, motility assays using both kinesin-2 and dynein-2 showed that the velocity of kinesin-2 was only minimally affected by inactive dynein-2, whereas an unstacked, active dynein-2 mutant conferred resistance against kinesin-2 (Toropova et al., 2017). *In vivo* support for dynein-2 auto-inhibition came from an analysis of IFT trains by using cryo-electron tomography in *Chlamydomonas* (Jordan et al., 2018). In this study, the anterograde trains were observed as densely packed and ordered structures composed of three repeats of approximately of 6, 11 and 18 nm, which were assigned to IFT-B, IFT-A and dynein-2 respectively. Notably, dynein-2 appeared in a stacked (autoinhibited) conformation when interacting with anterograde trains, with its stalks oriented away from the microtubule, which is likely to further inhibit the motor.

Recent cryo-EM and cryo-electron tomography studies shed light on how dynein-2's subunits enable it to associate with anterograde IFT trains to travel to the ciliary tip. In particular, dynein-2's subcomplex of intermediate and light chains has at least two important roles. First, it brings two copies of the heavy chain together into a stable dimer with auto-inhibited motors domains (Toropova et al., 2019), which is likely a suitable state for loading onto anterograde trains at the ciliary base (Wingfield et al., 2017). Second, the intermediate and light chains contort the two copies of the heavy chain into different conformations within the tail (Toropova et al., 2019). This asymmetric architecture is tailored to the repeating structure of the anterograde IFT-B train: each dynein-2 complex spreads out over seven to eight IFT-B repeats, and is tightly packed with the neighbouring dynein-2 complexes along the train (Jordan et al., 2018; Toropova et al., 2019) An important question for future studies is to determine which subunits of the IFT-B complex interact with dynein-2 on the anterograde train, but molecular genetic studies have implicated IFT172 as important for dynein-2 targeting or turnaround the ciliary tip (Pedersen et al., 2005; Tsao and Gorovsky, 2008; Williamson et al., 2012).

The mechanism by which dynein-2 is repositioned to bind to the axoneme and switched to an active conformation at the tip remains one of the most intriguing questions in the field. Biochemical and genetic studies suggest that classical dynein-1 accessory factors such as dynactin (Reck-Peterson et al., 2018) are not involved in dynein-2 regulation (Asante et al., 2014; Roberts, 2018). One possibility is that IFT-A and IFT-B themselves regulate dynein-

2 activity and that the rearrangement of these large complexes during train disassembly and reassembly facilitates a conformational switch within dynein-2 to form an active complex at the ciliary tip (Yi et al., 2017). Because the intermediate and light chains stabilise the auto-inhibited conformation of dynein-2, they must either rearrange or dissociate to activate the motor at the ciliary tip (Pazour et al., 2000; Toropova et al., 2019). Post-translational modifications of dynein-2 of the IFT subunits might have a role in dynein-2 activation, but these are not yet well described. It is also possible that other, thus far unknown regulators, are involved in this process.

Ciliogenesis and cilia function in dynein-2 mutants

Mutants in the dynein-2 heavy chain in many model organisms, including *Chlamydomonas*, *C. elegans*, mouse and zebrafish, and cultured mammalian cells, present similar phenotypes with short cilia and bulbous ciliary tips (Adhiambo et al., 2005; May et al., 2005; Pazour et al., 1999a; Porter et al., 1999; Wicks et al., 2000). In both mice (Wu et al., 2017) and cultured human cells (Vuolo et al., 2018), loss of WDR34 is associated with severe ciliogenesis defects, but others have shown that ciliogenesis is only moderately impaired in WDR34 knock-out (KO) cells (Tsurumi et al., 2019). In contrast, WDR60 is required for correct retrograde trafficking, but is dispensable for extending the ciliary axoneme in cultured human cells (Asante et al., 2014; Hamada et al., 2018; Vuolo et al., 2018). Moreover, fibroblasts from affected individuals with mutations in WDR60 still extend the ciliary axoneme, but the percentage of ciliated cells is variable (McInerney-Leo et al., 2013). Similar phenotypes with normal cilia length and a moderate reduction in cilia number were observed in TCTEX1D2 mutant fibroblasts from affected individual with short rib–polydactyly syndromes (SRPS) (Schmidts et al., 2015) or in TCTEX1D2-KO cells (Hamada et al., 2018).

Although defects in DYNC2LI1 do not completely abolish cilia extension, its mutation is associated with a ciliary accumulation of IFT proteins and defects in cilia length regulation, as observed in patient fibroblasts (Kessler et al., 2015; Taylor et al., 2015). Moreover, DYNC2LI1 appears to play a critical role in the stability of the dynein-2 complex in *Chlamydomonas* (Hou et al., 2004; Reck et al., 2016). These variations in phenotype could result from low level expression or, in some cases of genome engineering, expression of truncated proteins, leading to retention of partial function. Furthermore, loss of one subunit may affect the overall stability of the complex as has been seen for WDR34 and WDR60 KO. This outcome has also been clearly described for mice lacking the transcription factor ASCIZ (ATMIN) which have a severely reduced expression of the LC8 light chain,

DYNLL1, which results in partial depletion other dynein-2 subunits (King et al., 2019). Overall, full dynein-2 function does not appear to be absolutely required for ciliogenesis *per se*, but is needed to maintain the overall structure, including length control, and for core ciliary signalling functions.

Dynein-2 and the ciliary transition zone

New insights into IFT trafficking recently revealed an unexpected role for IFT-A and dynein-2 in maintaining compartmentalization of the transition zone (TZ) and thus of the ciliary structure in *C. elegans* and human cells. The TZ consist of a densely packed domain containing multiple proteins that are assembled in a tightly regulated process (see Box 1 and poster). The hierarchy of TZ assembly has been extensively described in several organisms and presents some common features in different models (reviewed in (Goncalves and Pelletier, 2017)). Super-resolution imaging and electron microscopy have resolved a map that defines the localization of distinct modules of the TZ (see poster). CEP290 (centrosomal protein 290 kDa) lies at the core of the TZ base and facilitates the assembly of other TZ components (Yang et al., 2015). RPGRIP1L ((retinitis pigmentosa GTPase regulator interacting protein 1-like; also called MKS-5 (Meckel syndrome type 5)) is a core component of *C. elegans* and vertebrate TZs (Li et al., 2016; Wiegering et al., 2018) that localizes distally to CEP290 and adjacent to the TZ microtubules. The NPHP (nephronophthisis) module links the CEP290 core to the MKS module that includes MKS1 (Meckel syndrome type 1), TCTN1 (Tectonic-1), TCTN2 (Tectonic-2), as well as several membrane proteins including TMEM67 (transmembrane protein 67) (Awata et al., 2014; Dean et al., 2016; Goncalves and Pelletier, 2017; Schouteden et al., 2015; Wang et al., 2013). This organization is also supported by proteomic mapping of the base of the cilium (Gupta et al., 2015). The TZ links the axonemal microtubules to the ciliary membrane and acts to gate entry and exit of proteins and lipids to the cilium. As such, it serves a vital function in the compartmentalization of ciliary signalling.

Recent data showed that dynein-2 is important to maintain the structure and integrity of the TZ. Loss of dynein-2 intermediate chains WDR34 and WDR60 caused a disruption of TZ composition in cultured human cells (Jensen et al., 2018; Vuolo et al., 2018), and a temperature-sensitive mutant showed that dynein-2 is required for TZ assembly and gating function in C. elegans (Jensen et al., 2018). In particular, the studies in human cells showed a distal extension of the RPGRIP1L domain of the TZ and a reduction of the TMEM67 area, whereas other TZ components, such as TCTN1 and CEP290, were not

affected. Interestingly, knockout of WDR34 and WDR60 was also associated with mislocalisation of several ciliary membrane proteins and IFT components, suggesting a defect in the entry and/or export mechanism that is regulated by the TZ (Vuolo et al., 2018). Consistent with these data, the temperature-sensitive mutation in the dynein-2 heavy chain resulted in a defective TZ composition in *C. elegans* (Jensen et al., 2018). Notably, at the restrictive temperature, some TZ components, such as NPHP4 (nephrocystin 4), CEP290 and MKS6 (Meckel Syndrome, Type 6), were mislocalised to a more distal region of the cilium. Furthermore, disruption of the TZ resulted in the ectopic localization of two different basal body proteins,TRAM1 (Translocating Chain-Associating Membrane Protein) and RPI2 (human retinitis pigmentosa-2 ortholog), in the ciliary axoneme (Jensen et al., 2018), suggesting a defect in the 'ciliary gate' formed by the TZ. Interestingly, proper TZ organisation was restored at permissive temperature, indicating that maintenance of TZ integrity is an active process that requires dynein-2.

It is uncertain how dynein-2 mediates TZ assembly, but this might involve its association with the IFT-A complex (Scheidel and Blacque, 2018). Analysis of IFT-A mutants indicated that IFT-A components play different roles in cilia entry and/or export of TZ components in the cilia in C. elegans. According to this model, core subunits of IFT-A (e.g. IFT140) promote entry of TZ proteins into cilia, whereas its non-core subunits (IFT121, IFT139, IFT43) regulate ciliary export. Consistent with observations in dynein-2 KO-cells (Vuolo et al., 2018), the key TZ component RPGRIPL1 is mislocalised in IFT-A mutants. Although the cilia from both IFT-A and dynein-2 mutants show a mislocalisation of several TZ proteins, no major defects are observed in the overall architecture of the TZ as determined by electron microscopy (Jensen et al., 2018). High-resolution views of the structure and dynamics of the TZ's components may help to elucidate its gating function and dependence on IFT-A and dynein-2.

Human diseases associated with defects in dynein-2 function

Defects in cilia formation and function lead to human pathologies, collectively termed ciliopathies (Reiter and Leroux, 2017). Mutations in dynein-2 are associated with a group of ciliopathies called 'skeletal ciliopathies' that are described as dysplasia (SRTD) with or without polydactyly (Huber and Cormier-Daire, 2012). The phenotypes related to skeletal ciliopathies include craniofacial abnormalities, short stature, shortened ribs, brachydactyly, and polydactyly. The skeletal phenotype can appear in association with defects in other organs, with retinal and kidney abnormalities as the most common symptoms observed

outside the skeletal system (Huber and Cormier-Daire, 2012). The skeletal abnormalities observed in some forms of SRTD patients are most likely related to defects in signalling pathways during embryonic development, including hedgehog (Hh), which requires cilia (Huangfu et al., 2003). In this context, cilia are particularly important to ensure correct Hh signalling during bone formation, and defects in dynein-2 result in the mislocalisation of Smoothened, a key component of Hh signalling, to cilia (May et al., 2005; Tsurumi et al., 2019; Vuolo et al., 2018; Wu et al., 2017). In recent years, whole exome-sequencing has enabled the identification of new mutations involved in skeletal ciliopathies, with the most common mutations affecting DYNC2H1 (Badiner et al., 2017; Cossu et al., 2016; Dagoneau et al., 2009; Merrill et al., 2009; Schmidts et al., 2013a). Moreover, mutations in WDR34 (Huber et al., 2013; Schmidts et al., 2013b), WDR60 (Cossu et al., 2016; McInerney-Leo et al., 2013), DYNC2LI1 (Kessler et al., 2015; Taylor et al., 2015), and TCTEX1D2 (Gholkar et al., 2015; Schmidts et al., 2015) have been also associated with SRTD, and a conditional KO of DYNLL1 in mouse limb mesoderm resulted in bone shortening, similar to that observed in SRTD patients (King et al., 2019). A comprehensive review of dynein-2 genes associated with skeletal ciliopathies has been recently published (Schmidts and Mitchison, 2018).

Conclusions

While we know much about the composition of the dynein-2 motor, its interactions, and now even have a structure of the dynein-2 complex, there is still much to be determined. A question for both mechanistic and clinical studies is how defects in dynein-2 relate to anterograde and retrograde trafficking. The tight co-assembly of dynein-2 with IFT-B trains defines its crucial position in anterograde IFT trains (Jordan et al., 2018; Toropova et al., 2019). Understanding the role of dynein-2 in maintaining a functional cilium and coordinating different signalling pathways, notably Hh, will likely help us to understand the contributions of dynein-2 and cilia in and skeletogenesis. Open questions include how, at the atomic level, dynein-2 co-assembles with IFT complexes at the ciliary base, and how its entry into the cilium is gated. It is also unclear what triggers the disassembly of anterograde kinesin-2-driven IFT trains at the ciliary tip, how retrograde trains - driven by active dynein-2 - are formed, or why dynein-2 is used to actively transport kinesin-2 to the ciliary base in vertebrate cilia (Broekhuis et al., 2014; Williams et al., 2014) when diffusion appears to be sufficient in *Chlamydomonas* (Chien et al., 2017; Engel et al., 2012).

Intensive and integrated efforts combining biochemistry, structural biology, clinical genetics, cell and developmental biology will be required to address these challenges, giving an opportunity to fully understand the mechanism and functions of dynein-2 in cilia biology and to apply this knowledge to improve human health.

Competing interests

The authors declare no competing or financial interests.

Funding

L.V., A.G.M., A.J.R. and D.J.S. work on dynein-2 is funded by a collaborative grant from UK Research and Innovation-Biotechnology and Biological Sciences Research Council (UKRI-BBSRC, BB/S005390/1). Further work in D.J.S.'s laboratory on dynein-2 is supported by UKRI-BBSRC [BB/S013024/1] and in A.J.R.'s laboratory by UKRI-BBSRC [BB/S007202/1 and BB/P008348/1] and The Wellcome Trust and Royal Society [104196/Z/14/Z].

BOX 1: Primary and motile cilia

Cilia are microtubule-based structures, with an axoneme based on nine cylindrically arranged microtubule (MT) doublets. Primary (a.k.a. sensory) cilia are solitary structures on the cell surface and function as ‘antenna’ that transduce signals from the extracellular environment. Motile cilia are present on specialised cell types and function to drive the movement of fluids in multiciliated epithelia in vertebrates, the locomotion of sperm, and the motility of many unicellular organisms. In addition to the nine microtubules doublets, motile cilia usually feature an additional central pair of MTs in the axoneme lumen (Mirvis et al., 2018). Axonemal dyneins generate the force to bend the axoneme in motile cilia (King and Sale, 2018). In all cilia and flagella, each microtubule doublet consists of A and B tubules, with the A tubule formed by 13 protofilaments and the B tubule formed by 10 protofilaments. While motile cilia typically present a 9+2 structure along the axoneme length, the structure of primary cilia is more variable. Recent electron tomography data indicate that in the primary cilium of several kidney cell lines, two of the microtubule doublets progressively shift toward the core of the axoneme at the region where the primary cilium starts to extend into the extracellular space, forming a 7+2 arrangement (Sun et al., 2019).

The structure of cilia includes a series of evolutionarily conserved subdomains, each defined by a specific cohort of proteins. The cilium extends from the basal body, formed by the mother centriole along with subdistal and distal appendages proteins. Transition fibres connect the basal body to the plasma membrane. Distal to the basal body is the transition zone (TZ), characterized by membrane-associated Y-shaped links. Transition fibres and the TZ compartment form a permeability barrier called the ‘ciliary gate’ that regulate ciliary protein composition (Jensen and Leroux, 2017) (see poster) .

BOX 2: The bidirectional intraflagellar transport system

IFT was first described in *Chlamydomonas reinhardtii,* where large particles moving in both directions along the length of the flagella were observed using differential interference contrast (DIC) microscopy (Kozminski et al., 1993). Subsequently, using time-lapse imaging of specifically-labelled proteins, IFT has been described in many model systems, including *Caenorhabditis elegans* (Orozco et al., 1999), *Tetrahymena thermophila* (Brown et al., 1999)*, Trypanosoma brucei* (Absalon et al., 2008) and vertebrate cells (Follit et al., 2006; Pazour et al., 2002; Pazour et al., 2000). IFT trafficking complexes called 'trains' comprise IFT-A and IFT-B subcomplexes, which mediate the interactions between the ciliary motors and cargo (see poster). The IFT-B complex is generally associated with anterograde trafficking; it is formed of a core subcomplex of 10 subunits (IFT88, -81, -74, -70, --56, 52, -46, -27, -25, and -22), a peripheral complex of six subunits (IFT172, -80, -57, -54, -38, and -20), and associates with the small GTPase RabL2 (Kanie et al., 2017). IFT-A, which is generally required for retrograde transport as well as the ciliary import of a variety of membrane proteins, includes IFT144, -140, - 139, -122, -121, and -43 (Taschner and Lorentzen, 2016), and associates with the cargo adapter TULP3 (Mukhopadhyay 2010). A further complex, the BBSome, associates with IFT trains to stabilise their assembly (Wei et al., 2012) and mediates retrograde membrane protein trafficking (Nachury and Mick, 2019). In *Chlamydomonas,* anterograde and retrograde IFT trains have been defined to move on the B and A tubules of the axonemal microtubule doublets, respectively (Stepanek and Pigino, 2016). While there are strong common features of IFT between model organisms, there are also key differences. In *Chlamydomonas,* kinesin-2 appears to mainly diffuse back to the ciliary base (Engel et al., 2012)*,* whereas, in metazoans, kinesin-2 motors appear to be recycled to the ciliary base predominantly by retrograde IFT (Mijalkovic et al., 2017; Signor et al., 1999; Vuolo et al., 2018; Williams et al., 2014). Interestingly, an additional dynein heavy chain, DHC-3, has been implicated in the formation of a subset of cilia in *C. elegans*, and DHC-3 was identified – together with the dynein-2 heavy chain - in genetic screens for anti-helminth resistance (Page, 2018). The deposited protein sequence for DHC-3 suggests it is a highly divergent dynein heavy chain that lacks ATP binding sites that is thus unlikely to function as a conventional motor.

References

Absalon, S., Blisnick, T., Kohl, L., Toutirais, G., Dore, G., Julkowska, D., Tavenet, A. and Bastin, P. (2008). Intraflagellar transport and functional analysis of genes required for flagellum formation in trypanosomes. *Mol Biol Cell* **19**, 929-44.

Adhiambo, C., Forney, J. D., Asai, D. J. and LeBowitz, J. H. (2005). The two cytoplasmic dynein-2 isoforms in Leishmania mexicana perform separate functions. *Mol Biochem Parasitol* **143**, 216-25.

Asante, D., Maccarthy-Morrogh, L., Townley, A. K., Weiss, M. A., Katayama, K., Palmer, K. J., Suzuki, H., Westlake, C. J. and Stephens, D. J. (2013). A role for the Golgi matrix protein giantin in ciliogenesis through control of the localization of dynein-2. *Journal of Cell Science* **126**, 5189-97.

Asante, D., Stevenson, N. L. and Stephens, D. J. (2014). Subunit composition of the human cytoplasmic dynein-2 complex. *Journal of Cell Science* **127**, 4774-87.

Awata, J., Takada, S., Standley, C., Lechtreck, K. F., Bellve, K. D., Pazour, G. J., Fogarty, K. E. and Witman, G. B. (2014). NPHP4 controls ciliary trafficking of membrane proteins and large soluble proteins at the transition zone. *Journal of Cell Science* **127**, 4714-27.

Badiner, N., Taylor, S. P., Forlenza, K., Lachman, R. S., University of Washington Center for Mendelian, G., Bamshad, M., Nickerson, D., Cohn, D. H. and Krakow, D. (2017). Mutations in DYNC2H1, the cytoplasmic dynein 2, heavy chain 1 motor protein gene, cause short-rib polydactyly type I, Saldino-Noonan type. *Clin Genet* **92**, 158-165.

Blisnick, T., Buisson, J., Absalon, S., Marie, A., Cayet, N. and Bastin, P. (2014). The intraflagellar transport dynein complex of trypanosomes is made of a heterodimer of dynein heavy chains and of light and intermediate chains of distinct functions. *Mol Biol Cell* **25**, 2620-33.

Broekhuis, J. R., Verhey, K. J. and Jansen, G. (2014). Regulation of cilium length and intraflagellar transport by the RCK-kinases ICK and MOK in renal epithelial cells. *PLoS One* **9**, e108470.

Brown, J. M., Marsala, C., Kosoy, R. and Gaertig, J. (1999). Kinesin-II is preferentially targeted to assembling cilia and is required for ciliogenesis and normal cytokinesis in Tetrahymena. *Mol Biol Cell* **10**, 3081-96.

Cao, M., Ning, J., Hernandez-Lara, C. I., Belzile, O., Wang, Q., Dutcher, S. K., Liu, Y. and Snell, W. J. (2015). Uni-directional ciliary membrane protein trafficking by a cytoplasmic retrograde IFT motor and ciliary ectosome shedding. *Elife* **4**.

Chien, A., Shih, S. M., Bower, R., Tritschler, D., Porter, M. E. and Yildiz, A. (2017). Dynamics of the IFT machinery at the ciliary tip. *Elife* **6**.

Cossu, C., Incani, F., Serra, M. L., Coiana, A., Crisponi, G., Boccone, L. and Rosatelli, M. C. (2016). New mutations in DYNC2H1 and WDR60 genes revealed by whole-exome sequencing in two unrelated Sardinian families with Jeune asphyxiating thoracic dystrophy. *Clin Chim Acta* **455**, 172-80.

Criswell, P. S., Ostrowski, L. E. and Asai, D. J. (1996). A novel cytoplasmic dynein heavy chain: expression of DHC1b in mammalian ciliated epithelial cells. *Journal of Cell Science* **109 (Pt 7)**, 1891-8.

Dagoneau, N., Goulet, M., Genevieve, D., Sznajer, Y., Martinovic, J., Smithson, S., Huber, C., Baujat, G., Flori, E., Tecco, L. et al. (2009). DYNC2H1 mutations cause asphyxiating thoracic dystrophy and short rib-polydactyly syndrome, type III. *American Journal of Human Genetics* **84**, 706-11.

Dean, S., Moreira-Leite, F., Varga, V. and Gull, K. (2016). Cilium transition zone proteome reveals compartmentalization and differential dynamics of ciliopathy complexes. *Proc Natl Acad Sci U S A* **113**, E5135-43.

Engel, B. D., Ishikawa, H., Wemmer, K. A., Geimer, S., Wakabayashi, K., Hirono, M., Craige, B., Pazour, G. J., Witman, G. B., Kamiya, R. et al. (2012). The role of retrograde intraflagellar transport in flagellar assembly, maintenance, and function. *J Cell Biol* **199**, 151-67.

Follit, J. A., Tuft, R. A., Fogarty, K. E. and Pazour, G. J. (2006). The intraflagellar transport protein IFT20 is associated with the Golgi complex and is required for cilia assembly. *Mol Biol Cell* **17**, 3781-92.

Gholkar, A. A., Senese, S., Lo, Y. C., Capri, J., Deardorff, W. J., Dharmarajan, H., Contreras, E., Hodara, E., Whitelegge, J. P., Jackson, P. K. et al. (2015). Tctex1d2 associates with short-rib polydactyly syndrome proteins and is required for ciliogenesis. *Cell Cycle* **14**, 1116-25.

Gibbons, B. H., Asai, D. J., Tang, W. J., Hays, T. S. and Gibbons, I. R. (1994). Phylogeny and expression of axonemal and cytoplasmic dynein genes in sea urchins. *Molecular Biology of the Cell* **5**, 57-70.

Goncalves, J. and Pelletier, L. (2017). The Ciliary Transition Zone: Finding the Pieces and Assembling the Gate. *Mol Cells* **40**, 243-253.

Grissom, P. M., Vaisberg, E. A. and McIntosh, J. R. (2002). Identification of a novel light intermediate chain (D2LIC) for mammalian cytoplasmic dynein 2. *Molecular Biology of the Cell* **13**, 817-29.

Gupta, G. D., Coyaud, E., Goncalves, J., Mojarad, B. A., Liu, Y., Wu, Q., Gheiratmand, L., Comartin, D., Tkach, J. M., Cheung, S. W. et al. (2015). A Dynamic Protein Interaction Landscape of the Human Centrosome-Cilium Interface. *Cell* **163**, 1484-99.

Hamada, Y., Tsurumi, Y., Nozaki, S., Katoh, Y. and Nakayama, K. (2018). Interaction of WDR60 intermediate chain with TCTEX1D2 light chain of the dynein-2 complex is crucial for ciliary protein trafficking. *Mol Biol Cell* **29**, 1628-1639.

Hao, L., Efimenko, E., Swoboda, P. and Scholey, J. M. (2011). The retrograde IFT machinery of C. elegans cilia: two IFT dynein complexes? *PLoS One* **6**, e20995.

Hou, Y., Pazour, G. J. and Witman, G. B. (2004). A dynein light intermediate chain, D1bLIC, is required for retrograde intraflagellar transport. *Mol Biol Cell* **15**, 4382-94.

Huangfu, D., Liu, A., Rakeman, A. S., Murcia, N. S., Niswander, L. and Anderson, K. V. (2003). Hedgehog signalling in the mouse requires intraflagellar transport proteins. *Nature* **426**, 83-7.

Huber, C. and Cormier-Daire, V. (2012). Ciliary disorder of the skeleton. *Am J Med Genet C Semin Med Genet* **160C**, 165-74.

Huber, C., Wu, S., Kim, A. S., Sigaudy, S., Sarukhanov, A., Serre, V., Baujat, G., Le Quan Sang, K. H., Rimoin, D. L., Cohn, D. H. et al. (2013). WDR34 Mutations that Cause Short-Rib Polydactyly Syndrome Type III/Severe Asphyxiating Thoracic Dysplasia Reveal a Role for the NF-kappaB Pathway in Cilia. *American Journal of Human Genetics* **93**, 926-31.

Jensen, V. L., Lambacher, N. J., Li, C., Mohan, S., Williams, C. L., Inglis, P. N., Yoder, B. K., Blacque, O. E. and Leroux, M. R. (2018). Role for intraflagellar transport in building a functional transition zone. *EMBO Rep* **19**.

Jensen, V. L. and Leroux, M. R. (2017). Gates for soluble and membrane proteins, and two trafficking systems (IFT and LIFT), establish a dynamic ciliary signaling compartment. *Curr Opin Cell Biol* **47**, 83-91.

Jordan, M. A., Diener, D. R., Stepanek, L. and Pigino, G. (2018). The cryo-EM structure of intraflagellar transport trains reveals how dynein is inactivated to ensure unidirectional anterograde movement in cilia. *Nat Cell Biol* **20**, 1250-1255.

Kanie, T., Abbott, K. L., Mooney, N. A., Plowey, E. D., Demeter, J. and Jackson, P. K. (2017). The CEP19-RABL2 GTPase Complex Binds IFT-B to Initiate Intraflagellar Transport at the Ciliary Base. *Dev Cell* **42**, 22-36 e12.

Kessler, K., Wunderlich, I., Uebe, S., Falk, N. S., Giessl, A., Brandstatter, J. H., Popp, B., Klinger, P., Ekici, A. B., Sticht, H. et al. (2015). DYNC2LI1 mutations broaden the clinical spectrum of dynein-2 defects. *Sci Rep* **5**, 11649.

King, A., Hoch, N. C., McGregor, N. E., Sims, N. A., Smyth, I. M. and Heierhorst, J. (2019). Dynll1 is essential for development and promotes endochondral bone formation by regulating intraflagellar Dynein function in primary cilia. *Hum Mol Genet*.

King, S. M., Barbarese, E., Dillman, J. F., 3rd, Benashski, S. E., Do, K. T., Patel-King, R. S. and Pfister, K. K. (1998). Cytoplasmic dynein contains a family of differentially expressed light chains. *Biochemistry* **37**, 15033-41.

King, S. M. and Sale, W. S. (2018). Fifty years of microtubule sliding in cilia. *Mol Biol Cell* **29**, 698-701.

Kollmar, M. (2016). Fine-Tuning Motile Cilia and Flagella: Evolution of the Dynein Motor Proteins from Plants to Humans at High Resolution. *Mol Biol Evol* **33**, 3249-3267.

Kozminski, K. G., Johnson, K. A., Forscher, P. and Rosenbaum, J. L. (1993). A motility in the eukaryotic flagellum unrelated to flagellar beating. *Proc Natl Acad Sci U S A* **90**, 5519-23.

Li, C., Jensen, V. L., Park, K., Kennedy, J., Garcia-Gonzalo, F. R., Romani, M., De Mori, R., Bruel, A. L., Gaillard, D., Doray, B. et al. (2016). MKS5 and CEP290 Dependent Assembly Pathway of the Ciliary Transition Zone. *PLoS Biol* **14**, e1002416.

Li, W., Yi, P. and Ou, G. (2015). Somatic CRISPR-Cas9-induced mutations reveal roles of embryonically essential dynein chains in Caenorhabditis elegans cilia. *J Cell Biol* **208**, 683-92.

May, S. R., Ashique, A. M., Karlen, M., Wang, B., Shen, Y., Zarbalis, K., Reiter, J., Ericson, J. and Peterson, A. S. (2005). Loss of the retrograde motor for IFT disrupts localization of Smo to cilia and prevents the expression of both activator and repressor functions of Gli. *Developmental Biology* **287**, 378-89.

McInerney-Leo, A. M., Schmidts, M., Cortes, C. R., Leo, P. J., Gener, B., Courtney, A. D., Gardiner, B., Harris, J. A., Lu, Y., Marshall, M. et al. (2013). Short-rib polydactyly and Jeune syndromes are caused by mutations in WDR60. *Am J Hum Genet* **93**, 515-23.

Merrill, A. E., Merriman, B., Farrington-Rock, C., Camacho, N., Sebald, E. T., Funari, V. A., Schibler, M. J., Firestein, M. H., Cohn, Z. A., Priore, M. A. et al. (2009). Ciliary abnormalities due to defects in the retrograde transport protein DYNC2H1 in short-rib polydactyly syndrome. *Am J Hum Genet* **84**, 542-9.

Mijalkovic, J., Prevo, B., Oswald, F., Mangeol, P. and Peterman, E. J. (2017). Ensemble and single-molecule dynamics of IFT dynein in Caenorhabditis elegans cilia. *Nat Commun* **8**, 14591.

Mikami, A., Tynan, S. H., Hama, T., Luby-Phelps, K., Saito, T., Crandall, J. E., Besharse, J. C. and Vallee, R. B. (2002). Molecular structure of cytoplasmic dynein 2 and its distribution in neuronal and ciliated cells. *Journal of Cell Science* **115**, 4801-8.

Mirvis, M., Stearns, T. and James Nelson, W. (2018). Cilium structure, assembly, and disassembly regulated by the cytoskeleton. *Biochem J* **475**, 2329-2353.

Nachury, M. V. and Mick, D. U. (2019). Establishing and regulating the composition of cilia for signal transduction. *Nat Rev Mol Cell Biol* **20**, 389-405.

Orozco, J. T., Wedaman, K. P., Signor, D., Brown, H., Rose, L. and Scholey, J. M. (1999). Movement of motor and cargo along cilia. *Nature* **398**, 674.

Page, A. P. (2018). The sensory amphidial structures of Caenorhabditis elegans are involved in macrocyclic lactone uptake and anthelmintic resistance. *Int J Parasitol* **48**, 1035-1042.

Palmer, K. J., Hughes, H. and Stephens, D. J. (2009). Specificity of cytoplasmic dynein subunits in discrete membrane-trafficking steps. *Molecular Biology of the Cell* **20**, 2885-99.

Patel-King, R. S., Gilberti, R. M., Hom, E. F. and King, S. M. (2013). WD60/FAP163 is a dynein intermediate chain required for retrograde intraflagellar transport in cilia. *Mol Biol Cell* **24**, 2668-77.

Pazour, G. J., Baker, S. A., Deane, J. A., Cole, D. G., Dickert, B. L., Rosenbaum, J. L., Witman, G. B. and Besharse, J. C. (2002). The intraflagellar transport protein, IFT88, is essential for vertebrate photoreceptor assembly and maintenance. *J Cell Biol* **157**, 103-13.

Pazour, G. J., Dickert, B. L., Vucica, Y., Seeley, E. S., Rosenbaum, J. L., Witman, G. B. and Cole, D. G. (2000). Chlamydomonas IFT88 and its mouse homologue, polycystic kidney disease gene tg737, are required for assembly of cilia and flagella. *J Cell Biol* **151**, 709-18.

Pazour, G. J., Dickert, B. L. and Witman, G. B. (1999a). The DHC1b (DHC2) isoform of cytoplasmic dynein is required for flagellar assembly. *J Cell Biol* **144**, 473-81.

Pazour, G. J., Koutoulis, A., Benashski, S. E., Dickert, B. L., Sheng, H., Patel-King, R. S., King, S. M. and Witman, G. B. (1999b). LC2, the chlamydomonas homologue of the t complex-encoded protein Tctex2, is essential for outer dynein arm assembly. *Molecular Biology of the Cell* **10**, 3507-20.

Pazour, G. J., Wilkerson, C. G. and Witman, G. B. (1998). A dynein light chain is essential for the retrograde particle movement of intraflagellar transport (IFT). *J Cell Biol* **141**, 979-92.

Pedersen, L. B., Miller, M. S., Geimer, S., Leitch, J. M., Rosenbaum, J. L. and Cole, D. G. (2005). Chlamydomonas IFT172 is encoded by FLA11, interacts with CrEB1, and regulates IFT at the flagellar tip. *Curr Biol* **15**, 262-6.

Porter, M. E., Bower, R., Knott, J. A., Byrd, P. and Dentler, W. (1999). Cytoplasmic dynein heavy chain 1b is required for flagellar assembly in Chlamydomonas. *Mol Biol Cell* **10**, 693-712.

Raaijmakers, J. A. and Medema, R. H. (2014). Function and regulation of dynein in mitotic chromosome segregation. *Chromosoma* **123**, 407-22.

Reck-Peterson, S. L., Redwine, W. B., Vale, R. D. and Carter, A. P. (2018). The cytoplasmic dynein transport machinery and its many cargoes. *Nat Rev Mol Cell Biol* **19**, 382-398.

Reck, J., Schauer, A. M., VanderWaal Mills, K., Bower, R., Tritschler, D., Perrone, C. A. and Porter, M. E. (2016). The role of the dynein light intermediate chain in retrograde IFT and flagellar function in Chlamydomonas. *Mol Biol Cell* **27**, 2404-22.

Reiter, J. F. and Leroux, M. R. (2017). Genes and molecular pathways underpinning ciliopathies. *Nat Rev Mol Cell Biol* **18**, 533-547.

Roberts, A. J. (2018). Emerging mechanisms of dynein transport in the cytoplasm versus the cilium. *Biochem Soc Trans* **46**, 967-982.

Rompolas, P., Pedersen, L. B., Patel-King, R. S. and King, S. M. (2007). Chlamydomonas FAP133 is a dynein intermediate chain associated with the retrograde intraflagellar transport motor. *Journal of Cell Science* **120**, 3653-65.

Rosenbaum, J. L. and Witman, G. B. (2002). Intraflagellar transport. *Nat Rev Mol Cell Biol* **3**, 813-25.

Scheidel, N. and Blacque, O. E. (2018). Intraflagellar Transport Complex A Genes Differentially Regulate Cilium Formation and Transition Zone Gating. *Curr Biol* **28**, 3279-3287 e2.

Schmidt, H., Zalyte, R., Urnavicius, L. and Carter, A. P. (2015). Structure of human cytoplasmic dynein-2 primed for its power stroke. *Nature* **518**, 435-438.

Schmidts, M., Arts, H. H., Bongers, E. M., Yap, Z., Oud, M. M., Antony, D., Duijkers, L., Emes, R. D., Stalker, J., Yntema, J. B. et al. (2013a). Exome sequencing identifies DYNC2H1 mutations as a common cause of asphyxiating thoracic dystrophy (Jeune syndrome) without major polydactyly, renal or retinal involvement. *Journal of Medical Genetics* **50**, 309-23.

Schmidts, M., Hou, Y., Cortes, C. R., Mans, D. A., Huber, C., Boldt, K., Patel, M., van Reeuwijk, J., Plaza, J. M., van Beersum, S. E. et al. (2015). TCTEX1D2 mutations underlie Jeune asphyxiating thoracic dystrophy with impaired retrograde intraflagellar transport. *Nat Commun* **6**, 7074.

Schmidts, M. and Mitchison, H. M. (2018). 15 - Severe skeletal abnormalities caused by defects in retrograde intraflagellar transport dyneins. In *Dyneins (Second Edition)*, (ed. S. M. King), pp. 356-401: Academic Press.

Schmidts, M., Vodopiutz, J., Christou-Savina, S., Cortes, C. R., McInerney-Leo, A. M., Emes, R. D., Arts, H. H., Tuysuz, B., D'Silva, J., Leo, P. J. et al. (2013b). Mutations in the gene encoding IFT dynein complex component WDR34 cause Jeune asphyxiating thoracic dystrophy. *Am J Hum Genet* **93**, 932-44.

Schouteden, C., Serwas, D., Palfy, M. and Dammermann, A. (2015). The ciliary transition zone functions in cell adhesion but is dispensable for axoneme assembly in C. elegans. *J Cell Biol* **210**, 35-44.

Schroeder, C. M., Ostrem, J. M., Hertz, N. T. and Vale, R. D. (2014). A Ras-like domain in the light intermediate chain bridges the dynein motor to a cargo-binding region. *Elife* **3**, e03351.

Signor, D., Wedaman, K. P., Orozco, J. T., Dwyer, N. D., Bargmann, C. I., Rose, L. S. and Scholey, J. M. (1999). Role of a class DHC1b dynein in retrograde transport of IFT motors and IFT raft particles along cilia, but not dendrites, in chemosensory neurons of living Caenorhabditis elegans. *J Cell Biol* **147**, 519-30.

Stepanek, L. and Pigino, G. (2016). Microtubule doublets are double-track railways for intraflagellar transport trains. *Science* **352**, 721-4.

Sun, S., Fisher, R. L., Bowser, S. S., Pentecost, B. T. and Sui, H. (2019). Three-dimensional architecture of epithelial primary cilia. *Proc Natl Acad Sci U S A* **116**, 9370-9379.

Tanaka, Y., Zhang, Z. and Hirokawa, N. (1995). Identification and molecular evolution of new dynein-like protein sequences in rat brain. *Journal of Cell Science* **108 (Pt 5)**, 1883-93.

Taschner, M. and Lorentzen, E. (2016). The Intraflagellar Transport Machinery. *Cold Spring Harb Perspect Biol* **8**.

Taylor, S. P., Dantas, T. J., Duran, I., Wu, S., Lachman, R. S., University of Washington Center for Mendelian Genomics, C., Nelson, S. F., Cohn, D. H., Vallee, R. B. and Krakow, D. (2015). Mutations in DYNC2LI1 disrupt cilia function and cause short rib polydactyly syndrome. *Nat Commun* **6**, 7092.

Toropova, K., Mladenov, M. and Roberts, A. J. (2017). Intraflagellar transport dynein is autoinhibited by trapping of its mechanical and track-binding elements. *Nat Struct Mol Biol* **24**, 461-468.

Toropova, K., Zalyte, R., Mukhopadhyay, A. G., Mladenov, M., Carter, A. P. and Roberts, A. J. (2019). Structure of the dynein-2 complex and its assembly with intraflagellar transport trains. *Nat Struct Mol Biol* **26**, 823-829.

Tsao, C. C. and Gorovsky, M. A. (2008). Different effects of Tetrahymena IFT172 domains on anterograde and retrograde intraflagellar transport. *Mol Biol Cell* **19**, 1450-61.

Tsurumi, Y., Hamada, Y., Katoh, Y. and Nakayama, K. (2019). Interactions of the dynein-2 intermediate chain WDR34 with the light chains are required for ciliary retrograde protein trafficking. *Mol Biol Cell* **30**, 658-670.

Vuolo, L., Stevenson, N. L., Heesom, K. J. and Stephens, D. J. (2018). Dynein-2 intermediate chains play crucial but distinct roles in primary cilia formation and function. *Elife* **7**.

Wang, W. J., Tay, H. G., Soni, R., Perumal, G. S., Goll, M. G., Macaluso, F. P., Asara, J. M., Amack, J. D. and Tsou, M. F. (2013). CEP162 is an axoneme-recognition protein promoting ciliary transition zone assembly at the cilia base. *Nat Cell Biol* **15**, 591-601.

Wei, Q., Zhang, Y., Li, Y., Zhang, Q., Ling, K. and Hu, J. (2012). The BBSome controls IFT assembly and turnaround in cilia. *Nat Cell Biol* **14**, 950-7.

Wicks, S. R., de Vries, C. J., van Luenen, H. G. and Plasterk, R. H. (2000). CHE-3, a cytosolic dynein heavy chain, is required for sensory cilia structure and function in Caenorhabditis elegans. *Developmental Biology* **221**, 295-307.

Wickstead, B. and Gull, K. (2007). Dyneins across eukaryotes: a comparative genomic analysis. *Traffic* **8**, 1708-21.

Wiegering, A., Dildrop, R., Kalfhues, L., Spychala, A., Kuschel, S., Lier, J. M., Zobel, T., Dahmen, S., Leu, T., Struchtrup, A. et al. (2018). Cell type-specific regulation of ciliary transition zone assembly in vertebrates. *EMBO J* **37**.

Williams, C. L., McIntyre, J. C., Norris, S. R., Jenkins, P. M., Zhang, L., Pei, Q., Verhey, K. and Martens, J. R. (2014). Direct evidence for BBSome-associated intraflagellar transport reveals distinct properties of native mammalian cilia. *Nat Commun* **5**, 5813.

Williamson, S. M., Silva, D. A., Richey, E. and Qin, H. (2012). Probing the role of IFT particle complex A and B in flagellar entry and exit of IFT-dynein in Chlamydomonas. *Protoplasma* **249**, 851-6.

Wingfield, J. L., Mengoni, I., Bomberger, H., Jiang, Y. Y., Walsh, J. D., Brown, J. M., Picariello, T., Cochran, D. A., Zhu, B., Pan, J. et al. (2017). IFT trains in different stages of assembly queue at the ciliary base for consecutive release into the cilium. *Elife* **6**.

Wu, C., Li, J., Peterson, A., Tao, K. and Wang, B. (2017). Loss of dynein-2 intermediate chain Wdr34 results in defects in retrograde ciliary protein trafficking and Hedgehog signaling in the mouse. *Hum Mol Genet* **26**, 2386-2397.

Yang, T. T., Su, J., Wang, W. J., Craige, B., Witman, G. B., Tsou, M. F. and Liao, J. C. (2015). Superresolution Pattern Recognition Reveals the Architectural Map of the Ciliary Transition Zone. *Sci Rep* **5**, 14096.

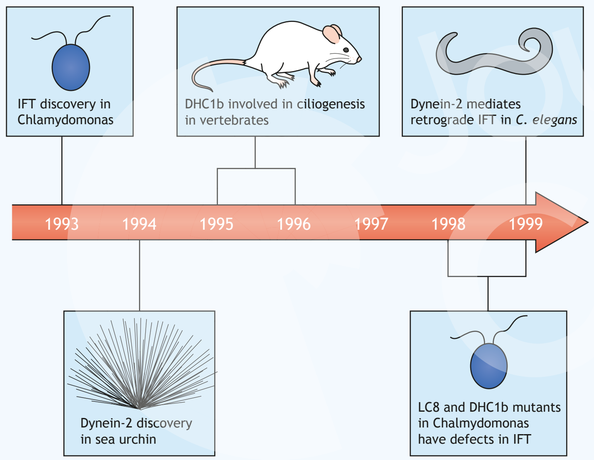
Journal of Cell Science

Cytoplasmic Dynein-2 at a Glance

Laura Vuolo, Nicola L. Stevenson, Aakash G. Mukhopadhyay, Anthony J. Roberts and David J. Stephens

The Company of Biologists

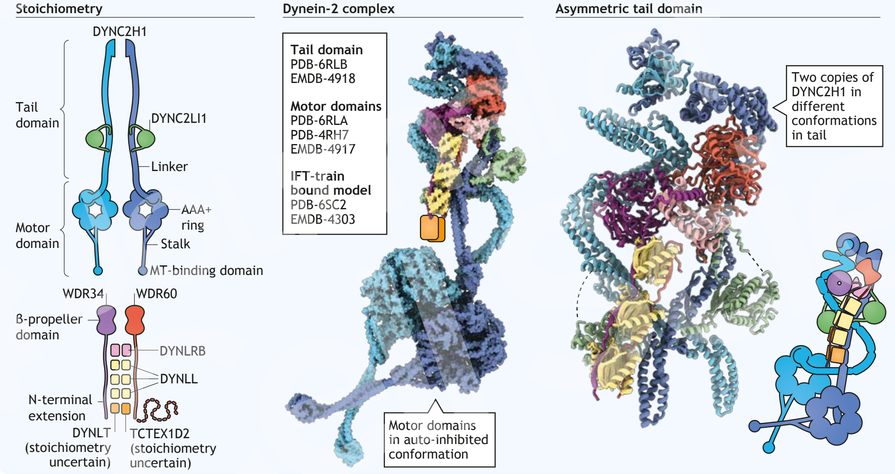
Discovery of dynein-2 and its involvement in IFT



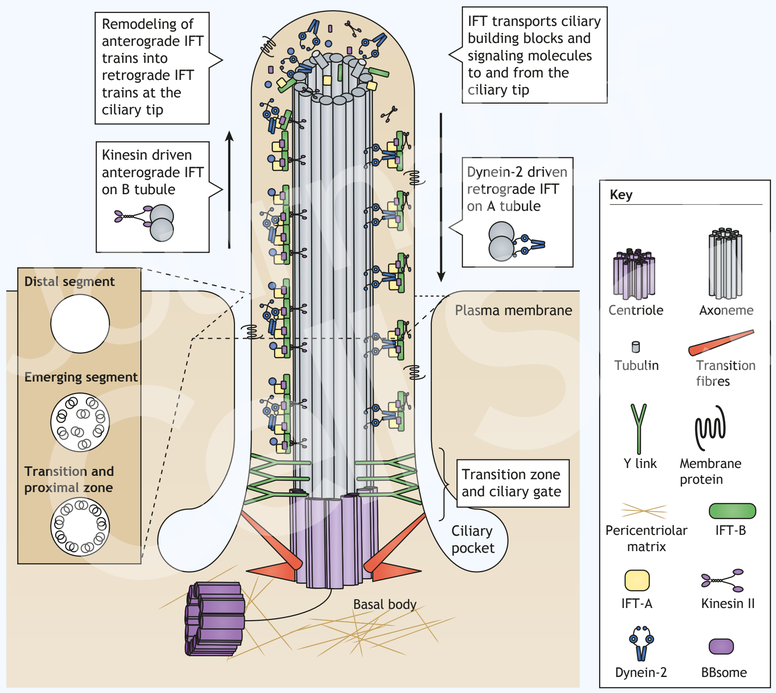
Subunit composition of dynein-2

Chain type	Alias	*H. sapiens*	*C. reinhardtii*	*C. elegans*	
Heavy	DHC2	DYNC2H1	DHC1b	CHE-3	1 Tail — Motor domain — 4,307 amino acids (*H. sapiens*)
Intermediate	WDR60	WDR60	D1bIC1 (FAP163)	Ambiguous	1 — β-propeller domain — 1066
	WDR34	WDR34	D1bIC2 (FAP133)	Ambiguous	1 — 536 — β-propeller domain
Light intermediate	LIC3	DYNC2LI1	D1bLIC	XBX-1	1 — 351 — Ras-like domain
Light	RB	DYNLRB1 DYNLRB2	LC7b LC7a	DYRB-1	1 — 96 — Also found in cytoplasmic dynein-1
	LC8	DYNLL1 DYNLL2	LC8	DLC-1	1 — 89 — Also found in cytoplasmic dynein-1
	TCTEX	DYNLT1 DYNLT3	Tctex1	DYLT-1 DYLT-3	1 — 113 — Also found in cytoplasmic dynein-1
	TCTEX1D2	TCTEX1D2	Tctex2b	DYLT-2	1 — 142

Structure of dynein-2

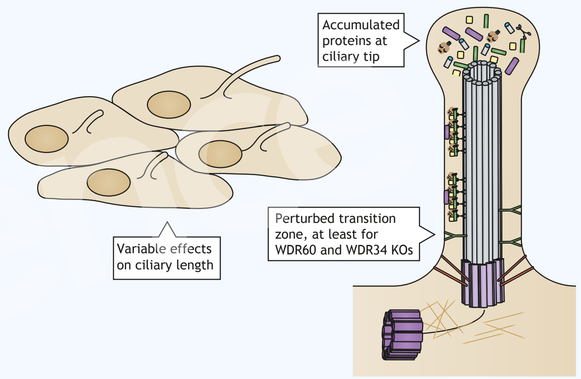


Role of dynein-2 in the bidirectional IFT system

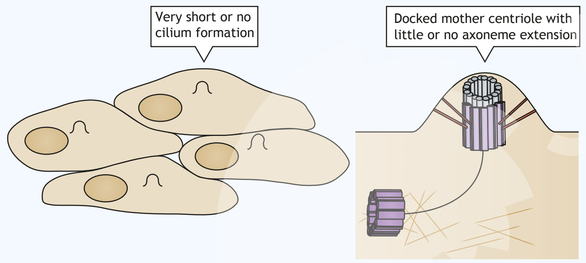


Impact of dynein-2 subunit mutants

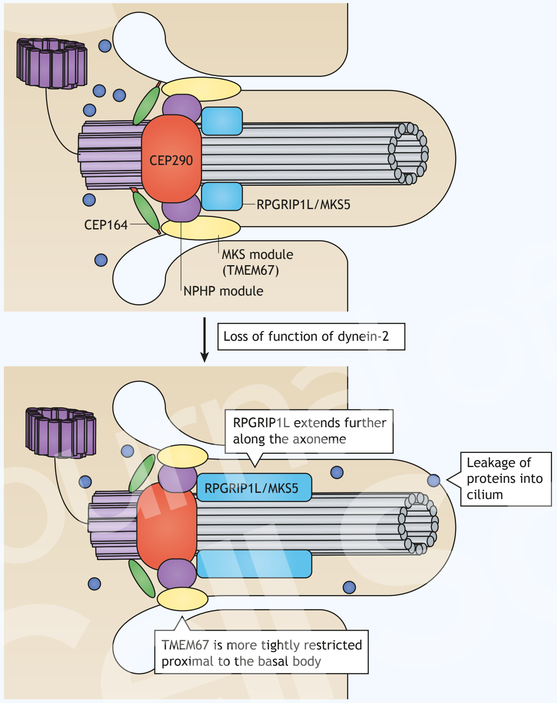
KO of dynein-2 specific, non-motor subunits (WDR34, WDR60, TCTEX1D2, LIC3)



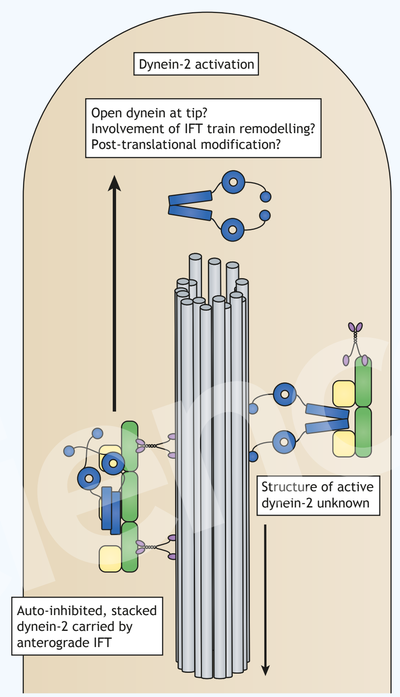
KO of heavy chain



Dynein-2 and the ciliary transition zone



Dynein-2 activation at ciliary tip



Abbreviations: ABC

doi: xxxxxxxxxxxxxxxxxx